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Toxicity and Repellency of Patchouli Oil and Patchouli Alcohol against Formosan Subterranean Termites *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae)

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Patchouli oil obtained from *Pogostemon cablin* (Blanco) Benth and its main constituent, patchouli alcohol, were tested for their repellency and toxicity against Formosan subterranean termites (*Coptotermes formosanus* Shiraki). Both were found to be toxic and repellent. Unusual tissue destruction was noted inside the exoskeleton of the termite after patchouli alcohol was topically applied to the dorsum.

KEYWORDS: ***Pogostemon cablin*; Formosan subterranean termites; repellent; insecticide.**

INTRODUCTION

Synthetic chemicals are currently used for the control of termites. However, the disadvantages of conventional termiticides such as the chlorinated hydrocarbons, organophosphates, and pyrethroids include residual toxicity and health hazards to humans and other nontarget species (*1*, *2*). Plant extracts have been used as insecticides at least from the time of ancient Rome. Some essential oils isolated by steam distillation from odoriferous plants are reported as repellents, fumigants, and contact insecticides (*3*−*6*). Recently, essential oils have received more attention as sources of useful insect-active compounds because of the necessity of finding safer insecticides. The main components in essential oils are monoterpenes, and related phenols include sesquiterpenes (*6*). Insecticidal essential oils already tested are monoterpenoids such as eugenol, geranoil, nerol, and citral (*5*, *7*). Some monoterpenoids have been found to be termiticidal (*3*, *8*).

Sesquiterpenoids are derived biosynthetically from three isoprene units and share farnesyl pyrophosphate as a common biosynthetic intermediate (*9*). There are many more sesquiterpenes known in nature than there are monoterpenoids. Vetiver oil, an essential oil, contains mostly sesquiterpenoids and their derivatives, some of which are repellent to flies and cockroaches (*10*). We reported that vetiver oil is also a repellent to Formosan subterranean termites, *Coptotermes formosanus* Shiraki, and that it disrupted termite tunneling behavior and food consumption (*11*, *12*).

In a search for more insect-active sesquiterpenes, we investigated patchouli oil, an essential oil obtained by steam distillation of the leaves of *Pogostemon cablin* (Blanco) Benth. (Labiatae) leaves. The oil is an important natural material in the perfumery and food industry and has been used in Asia historically to repel clothes moths and as a cold treatment (Akhila and Tewari, 1984). Patchouli oil is almost entirely composed of sesquiterpenes which contain eight different types of carbon skeletons (*13*). In this study, we report that patchouli oil and its major component, patchouli alcohol, are repellents and toxicants to Formosan subterranean termites. We also observed that the internal tissues were damaged after patchouli alcohol was applied topically to the termite integument.

METHODS AND MATERIALS

Patchouli (*Pogostemon cablin*) oil was purchased from The Good Scents Company (Oak Creek, WI).

Termites. Two carton nests (colonies B and E) of Formosan subterranean termites, *Coptotermes formosanus* Shiraki, were collected on April 24, 2002 in New Orleans, LA (B), and Lake Charles, LA (E). Termites were held in 250-L cans with pine used as a food source and kept at 24−26 °C. Moistened corrugated cardboard rolls were used to retrieve termites from the cans (*14*). Termites were gently knocked from the cardboard rolls into clean plastic trays (40 cm × 50 cm) and isolated from debris by allowing them to climb on moistened paper towels.

Constituents of Patchouli Oil. Gas chromatography−mass spectrometry (GC-MS) was performed on a Finnigan GCQ (Trace GC 2000 coupled with a Polaris MSD) as described by Zhu et al. (*11*). Patchoulol and other constituents were identified by searching standard mass spectra in the mass spectra library (XCalibur) and by published spectral data (*15*). Percentages of patchouli alcohol and other components in patchouli oil were determined by integrating GC peaks.

Purification of Patchouli Alcohol. One gram of patchouli oil was applied to a Silica (60 Å pore, 35−75 μm particle) column (2 cm × 30 cm), and the compounds in the oil were eluted with CH_3Cl. Each fraction was checked by thin-layer chromatography and GC-MS. The fraction containing patchouli alcohol was further purified by silica column chromatography, and its purity was determined by GC-MS.

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Repellency Test. The method for testing repellency was described previously (*16*). One milliliter of hot agar solution (1.0 g of agar in 100 mL of H_2O) was spread evenly in the bottom of a Petri dish (1 × 5 cm, 20 mL air) and allowed to cool. Blasting sand (fine, #4) was used after having been autoclaved for 30 min and oven dried. One-half of the Petri dish was covered with 1 g of treated sand. The other half was covered with 1 g of untreated sand.

Five concentrations (3.00, 6.25, 12.50, 25.00 and 50.00 μg/g sand) of each sample were tested to observe the minimum effective concentration (threshold value). The threshold value was calculated as μg of oil or chemical per g sand. Ten worker termites were randomly introduced into each Petri dish, and all Petri dishes were covered with a sheet of aluminum foil between observations to reduce possible effects of light. Two colonies (colony B and E) with 5 replicates of each were tested. The number of termites on the treated side was recorded at 1, 2, 3, 4, 5 and 24 h.

Tunneling, Paper Consumption, and Mortality. A three-chamber container was used for this assay (*11*). A small hole (1 cm diameter) was cut at the bottom of each of two inner walls. Fifty grams of untreated sand was added to the first chamber, and 50 g of treated sand was placed in the middle chamber. The third chamber contained a piece of weighted filter paper as a food source. Fifty workers and five soldier Formosan subterranean termites were placed on top of the sand in the first chamber (home chamber). The containers were covered with lids and kept in a dark incubator at 27−28 °C. Two colonies (colony B and E) with five replicates of each were tested. On the 14th day, each apparatus was dismantled and living termites were counted. Paper consumption was calculated as the difference between the weight of filter paper before and after the 14-day incubation. The tunnels termites constructed in the sand were recorded using a scanner for measurements of total tunnel lengths.

Assay for LD_{50} and LD_{90}. Twenty worker termites were placed in Petri dishes (60 mm diameter), and the dishes were placed on ice for 2 min. Patchouli oil or patchouli alcohol (0.2 μL) in ethanol was applied topically to the termite cuticle using a Hamilton PB 600−1 dispenser (Hamilton Company, Reno, NV 89520). Six concentrations (1.25, 2.5, 5.0, 10.0, 20.0, and 40.0 μg/termite) with five replicates for each of two colonies (B and E) were assayed. Ethanol (0.2 μL) was applied to the cuticle of 20 termites as a control. Treated termites were kept in Petri dishes with moist filter paper at 28 °C, and termite mortality was recorded at 24 h.

Behavior of the Treated Termites and Observation of the Damage to Tissues. Patchouli alcohol (0.2 μL, 20 μg) or ethanol (0.2 μL) was applied topically to 10 worker termites on the abdominal terga. The behavior of termites was observed with a videotape recorder. All termites were fixed with FAA solution (45% ethanol, 5% acetic acid, 2% Para formaldehyde) after 1 h exposure to patchouli alcohol or ethanol, and the fixed termites were examined under the light microscope (magnification: 15).

Data Analysis. For the repellency assay, the differences between the percentage of termites on untreated sand and an expected value were statistically analyzed by chi-square, using an expected value of 50% and a significance level of $p \leq 0.05$. The lethal concentrations of LD_{50} and LC_{90} were calculated with confidence limits of 95% by probit analysis using LeOra Software (*17*). Tunneling response, paper consumption, and mortality of termites were analyzed by ANOVA (18). Tukey's studentized range test was used to compare differences between treatment means.

RESULTS

Gas chromatography and mass spectra (GC-MS) of patchouli oil and purified patchouli alcohol are shown in **Figure 1A−C**. The oil consisted of about 40% patchouli alcohol with a second major compound being a-patchoulene (15%) (**Figure 1A**). The purity of patchouli alcohol after silica column chromatography was >90% (**Figure 1B**).

Patchouli oil and patchouli alcohol were repellent to Formosan subterranean termites as shown in **Table 1**. The minimum effective concentration (threshold value) of patchouli oil for colony B was higher than patchouli alcohol; it was the opposite for colony E. The repellency of patchouli oil and patchouli alcohol remained effective for all time periods tested.

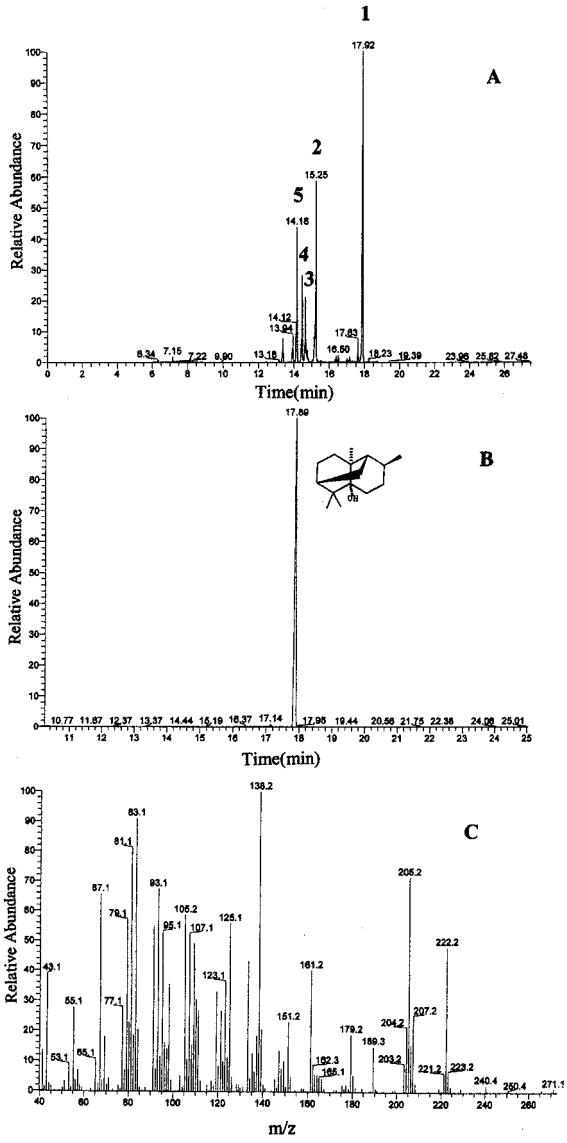

Figure 1. Gas chromatography of patchouli oil is shown in **A** and peak 1, 2, 3, 4 and 5 were patchouli alcohol, α-bulnesene, α-patchoulene, seychellen and α-guaiene respectively. GC-MS of purified patchouli alcohol are shown in (**B**) and (**C**).

Table 1. Repellency of Patchouli Oil and Patchouli Alcohol to Formosan Subterranean Termites[a]

		threshold value of repellency (μg/g of sand)			
treatment	colony	1 h	3 h	5 h	24 h
patchouli oil	B	25	25	50	50
	E	12.5	12.5	12.5	12.5
patchouli alcohol	B	3	3	6.25	12.5
	E	25	25	25	50

[a] Colony B: collected in New Orleans, LA, on April 24, 2002. Colony E: collected in Lake Charles, LA, on April 24, 2002. The compounds were significant repellent ($p < 0.005$) by chi-square analysis based on an expected 50% distribution between treated and untreated side in a Petri dish. Threshold value is the lowest effective concentration (μg/g of sand) for repellency.

Mean consumption of filter paper was decreased as patchouli oil (**Table 2**) and patchouli alcohol (**Table 3**) concentration increased ($F = 16.79$, $df = 5, 24$, $p < 0.0001$ and $F = 32.27$,

Table 2. Mean (±SD) of Paper Consumption, Percent Mortality, and Tunneling Length of Formosan Subterranean Termites after 14 Days Exposure of Patchouli Oil[a]

concentration of patchouli oil (μg/g sand)	weight loss of filter paper (mg)	termite mortality (%)	tunneling length (cm)
0	22.1 + 4.0 a	13.6 + 5.2 b	35.2 + 6.1 a
5	12.1 + 6.7 bc	15.6 + 11.1 b	39.0 + 8.1 a
10	16.2 + 11.0 ba	17.2 + 6.7 b	37.6 + 4.3 a
25	7.1 + 3.9 dc	33.2 + 6.1 b	27.0 + 12 a
50	2.76 + 1.6 d	28.8 + 4.6 b	10.0 + 5.9 b
100	0.0 + 0.0 d	62.0 + 20.0 a	0.0 + 0.0 b
	F = 16.79, df = 5, 24, P < 0.0001	F = 15.25, df = 5, 24, P < 0.0001	F = 58.31, df = 5, 24, P < 0.0001

[a] Colony B was collected in New Orleans, LA, on April 24, 2002. Tunneling length represents the tunneling activity of termites in the treated chamber. Means in the same column followed by the same letter are not significantly different using Tukey's studentized range test (p > 0.05).

Table 3. Mean (±SD) of Paper Consumption, Percent Mortality, and Tunneling Length of Formosan Subterranean Termites after 14 Days Exposure of Patchouli Alcohol[a]

concentration of patchouli alcohol (μg/g sand)	weight loss of filter paper (mg)	termite mortality (%)	tunneling length (cm)
0	22.1 + 4.0 a	13.6 + 5.2 c	35.2 + 6.0 b
5	20.0 + 2.7 a	16.0 + 4.0 c	46.0 + 10.6 a
10	16.1 + 9.3 a	22.4 + 2.6 b	24.6 + 4.8 c
25	0.0 + 0.0 b	97.6 + 5.4 a	0.0 + 0.0 d
50	0.0 + 0.0 b	100.0 + 0.0 a	0.0 + 0.0 d
100	0.0 + 0.0 b	100.0 + 0.0 a	0.0 + 0.0 d
	F = 32.27, df = 5, 24, P < 0.0001	F = 15.25, df = 5, 24, P < 0.0001	F = 58.31, df = 5, 24, P < 0.0001

[a] Colony B was collected in New Orleans, LA, on April 24, 2002. Tunneling length represents the tunneling activity of termites in the treated chamber. Means in the same column followed by the same letter are not significantly different using Tukey's studentized range test (p > 0.05).

df = 5, 24, p < 0.0001 respectively). Filter paper consumption was not observed when the concentration of patchouli oil was ≥100 μg/g of sand and that of patchouli alcohol was ≥25 μg/g of sand. Sand treated with patchouli oil or its alcohol resulted in a significant reduction in tunneling and feeding activity, and both were toxic to termites (**Tables 2** and **3**). The tunneling activity of termites treated with patchouli alcohol was lower than that with patchouli oil when the concentrations were >10 μg/g of sand. No tunneling activity in treated chambers was found at concentrations greater than 25 μg of patchouli alcohol per gram of sand or 100 μg of patchouli oil per gram of sand. Termites were able to tunnel in the untreated chambers (home compartment) at all concentrations of patchouli oil tested, but they were unable to tunnel in the home chambers if patchouli alcohol concentrations were ≥25 μg/g of sand. Termites in this condition exhibited "lingering behavior", that is, instead of digging in sand, termites remained clumped together on the surface or against a corner of the home compartment. This behavior was previously observed with components of vetiver oil (*12*). Termite mortality increased significantly as patchouli oil and patchouli alcohol concentration increased (F = 15.25, df = 5, 24, p < 0.0001 and F = 15.25, df = 5, 24, p < 0.0001, respectively). All termites were dead in 72 h at 50 mg of patchouli alcohol per gram of sand, while 28.8 ± 4.6% termites were dead at the same concentration of patchouli oil after 14 days. The mortality in the control was 13.6 ± 5.2% (**Table 2**). The LD_{50} (24 h) and LD_{90} (24 h) indicated that patchouli alcohol was twice as toxic to termites as patchouli oil (**Table 4**).

Table 4. Contact Toxicity of Patchouli Oil and Patchouli Alcohol[a]

colony	compound	LD_{50}	LD_{90}	slope ± SE
B	patchouli oil	11.6 (10.47–12.8)	24.84 (21.7–29.6)	4.05 ± 0.35
B	patchouli alcohol	5.33 (4.23–6.33)	11.74 (9.72–15.64)	3.74 ± 0.56
E	patchouli oil	7.52 (6.27–8.86)	14.62 (11.98–20.04)	4.43 ± 0.65
E	patchouli alcohol	3.8 (3.31–4.25)	6.22 (5.51–7.33)	6.0 ± 0.75

[a] Colony B: collected in New Orleans, LA on April 24, 2002. Colony E: collected in Lake Charles, LA on April 24, 2002. The lethal doses expressed as μg/termite, and the end-point mortality is obtained at 24 h treatment.

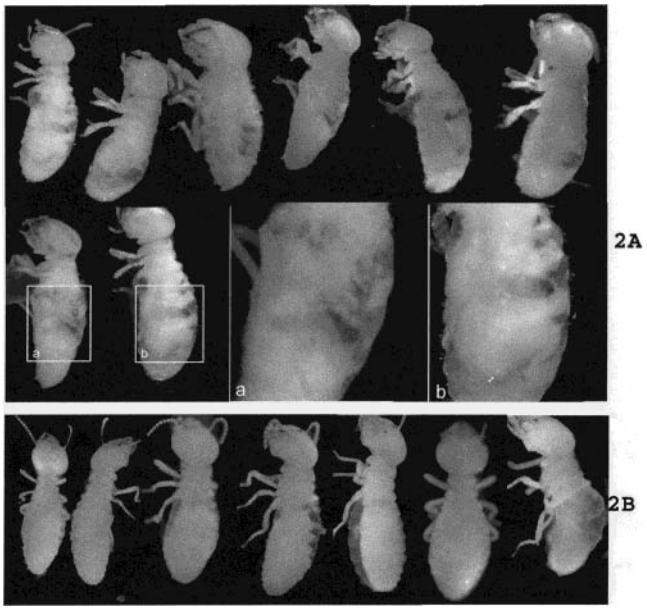

Figure 2. (**A**) Internal tissues of termites after topical contact with patchouli alcohol, and (**B**) termites which were only treated with ethanol. Magnification: 15 for the whole body (**A**, **B**) and 36 for the body in the panels (a, b).

Termites topically treated with patchouli alcohol began experiencing convulsions and tremors within a few minutes and became paralyzed, moribund, and were dead within 1 h. Eighty percent (8/10) of the treated termites were dead within 60 min, whereas the control termites (treated topically with ethanol) were all alive. The damage to termites caused by topical application of patchouli alcohol was observed under the microscope and is shown in **Figure 2A**. The exoskeleton of termites remained intact, but the internal tissue appeared dissolved and translucent. The most damaged area on the bodies was observed at the application site on the dorsal side where the patchouli alcohol was applied. The exoskeleton and internal tissue of control termites were not significantly changed (**Figure 2B**).

DISCUSSION

Our study demonstrated that patchouli oil and patchouli alcohol are both repellent and toxic to Formosan subterranean termites. The repellency threshold values for patchouli oil and patchouli alcohol differed between the colonies tested, but both remained effective for 24 h. Patchouli alcohol caused significantly greater termite mortality than patchouli oil and was about twice as toxic as patchouli oil based on LD_{50} and LD_{90} values. Since the threshold values for activity for patchouli oil and

patchouli alcohol differed between the colonies and the toxicity of patchouli alcohol is higher than that of patchouli oil in both colonies tested, we conclude that patchouli alcohol is primarily responsible for the toxicity of patchouli oil.

The acute topical toxicity of patchouli alcohol was partly characterized by the tissue damage of treated termites. Whether patchouli alcohol directly destroys cells or caused self-destruction of the tissue is not yet known. That the lesions appear localized at the point of the application site suggests that direct damage on the permeable membranes.

Monoterpenes in several essential oils have been shown to be compeititve inhibitors of acetyl-cholinesterase (AChE) (*19*, *20*). Some essential oil components are antagonists of octopamine receptors (*21*). Toxicity can be profoundly influenced by the ability of a toxicant to penetrate membranes and reach the target site (*6*). Certain essential oils are reported to exhibit a neurotoxic mode of action, irrespective of the route of administration (oral, topical, thorugh fumigation or from contacting residues on surfaces) (*6*). The common symptoms of a neurotoxic mode of action include hyperactivity, convulsions, tremors and paralysis. Our observations of the termite behavior suggest that high doses of patchouli oil (40 μg topical) or patchouli alcohol (20 μg, topical) have a neurotoxic mode of action toward termites. It is possible that the toxicity of patchouli oil and patchouli alcohol have multiple actions against termites including neurotoxicity, penetrating and "dissolving" cuticles and membranes, and causing internal tissue damage.

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